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Research Article

A study on antibacterial effect of grape seed extracts in common clinical and drug resistant isolates

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ABSTRACT

Background: Grape seeds are proposed to have antimicrobial activity, antioxidant effect and various other benefits to mankind. A study was done to assess the antibacterial effect of grape seed extract against common clinical isolates and drug resistant pathogenic strains.
Methods: Grape seed extract prepared was investigated for its antibacterial effect against 65 bacterial isolates obtained from clinical specimens by agar well diffusion assay and the results were compared with routinely used antibiotics namely, Gentamicin for the common clinical isolates, Vancomycin for MRSA strains and Amikacin for ESBL organisms respectively.
Results: Grape seed extract produced moderate zone of inhibition ranging between 11-15 mm among the 35 test common clinical isolates namely *Staphylococcus aureus*, *Escherichia coli*, *Klebsiella* sp and *Pseudomonas aeruginosa*. *E.coli* showed the highest susceptibility with zone ranging from 12-14 mm with increasing concentration of the extract starting from2 mg/ml to the highest being 20 mg/ml. Among the 30 drug resistant pathogenic strains like MRSA and ESBL producing organisms, the grape seed extract was found to be effective against 3 out of the 10 of MRSA and 2 out of the 10 of ESBL-*E.coli* at the highest concentration of 20 mg/ml. However, ESBL producing *Klebsiella* species were found to be resistant even to the highest concentration of the extract.
Conclusions: The results provide evidence that the grape seed extract could be a potential antibacterial agent and this effect can further be made evident with improved methodologies.

Keywords: Grape seed extract, ESBL, MRSA

INTRODUCTION

A wide variety of antibiotics are commonly used for the treatment of infections caused by bacteria. In recent years, multiple drug resistance, a threat to mankind has caused an urgent need for the search of innovative ways to control bacterial pathogens. Hence, natural antibiotics are in process of being discovered as alternative to synthetic products. Grape seeds used in herbal medicine and as dietary supplements are proposed to provide antimicrobial activity, antioxidant effect, wound healing and prevention of cardiovascular diseases. The polyphenolic fractions and gallic acid derivatives are reported to have antibacterial property.[1] The advantage of grape seed extract are numerous since it is natural, cost efficient and readily available. With these factors in mind, a study was done to assess the antibacterial effectiveness of grape seed extract against common clinical isolates and drug resistant pathogens. This study was done under ICMR - STS project.

METHODS

Extraction of grape seed extract

Institutional ethical committee approval was obtained prior to the study. Informed consent was obtained from the patients. 65 bacterial strains including MRSA and ESBL producing organisms were isolated from clinical specimens such as blood, urine, sputum, tissue and swabs. Fully ripe grapes were crushed and seeds were separated and added to a conical flask containing 100ml of ethanol and stirred well. The flask was left aside for 48 hours and occasionally stirred. The contents of the flask were filtered through Whatman no.1 and evaporated to dryness in oven at 50^{o}C. After the grape seed powder had been obtained, different concentrations of grape seed extract (2, 5,10,15, 20 mg/ml) were prepared by mixing the grape seed powder with dimethyl sulfoxide (DMSO).[2]

Isolation of bacteria from clinical specimens

The specimens were processed in culture plates and the pathogens were isolated and identified by standard biochemical tests. Mueller-Hinton agar (MHA) was used to determine the antibacterial effect of grape seed extract and the routinely used antibiotics, gentamicin for the common clinical isolates, vancomycin for MRSA strains and amikacin for ESBL organisms were used for comparison.[3,4]

In-vitro antibacterial study

The inoculum was spread on the MHA. Using sterile cork borer, wells of 8 mm diameter were punched in all the inoculated plates.100 µl of different concentrations of grape seed extract were added to the labelled well. DMSO was used as negative control and the standard antibiotics were used as positive control. Plates were incubated for 24 hours at 37°C. After 24 hours the presence or absence of inhibition zones was examined. The zone of inhibition of grape seed extract as well as standard antibiotic discs Gentamicin for *Escherichia coli*, *Klebsiella* sp, *Pseudomonas* & *Staphylococcus* and Vancomycin and Amikacin for strains like MRSA and ESBL producing organisms were measured and compared using a diameter scale. All the data were analyzed statistically with standard SPSS software (IBM SPSS). The results were expressed as mean±SD. Significant value was found when compared the effectiveness of the extract between the common clinical isolates and drug resistant strains the significant value was found

RESULTS

The grape seed extract showed bactericidal activity against all the selected 35 gram positive and gram negative organism which were isolated from the clinical samples. The zone of inhibition showed by the grape seed extract against the test bacteria ranged from 11-14 mm, as observed from Table 1. *Escherichia coli* showed the highest susceptibility with zones ranging from 12-14 mm with increasing concentration of the extract. *Pseudomonas aeruginosa* showed minimum growth inhibitory effects even at a concentration of 20 mg/ml of the extract. Therefore, on comparing the zones produced by the extract and the routinely used antibiotic gentamicin, grape seed extract had proved to be considerably efficient. As observed from Table 2, out of the total 30 assessed samples, the grape seed extract was found to be effective against 3 out of the 10 samples of MRSA and 2 out of the 10 samples of ESBL-*E.coli* at the highest concentration of 20 mg/ml with mild zones of inhibition 4.0±6.5 and 2.7±5.7 respectively. However, ESBL producing *Klebsiella* species were found to be resistant even to the highest concentration of the extract.

Figure 1: Showing the susceptibility of *Staphylococcus aureus* to varying concentrations of grape seed extract. The zones of inhibition is found to increase in size progressively to increased concentration of the extract.

Figure 2: Showing the susceptibility of MRSA species to the highest concentration of the extract being 20 mg/ml. The results are compared with the zone of inhibition obtained by using vancomycin as positive control and DMSO as negative control.

Table 1: Antibacterial effect of grape seed extract against the common clinical isolates.

S. No	Bacterial species	No.of isolates	Zone of inhibition(mm)						
			Concentration of grape seed extract(mg/ml)					Control	
								+VE control	-VE control
			2	5	10	15	20	Gentamicin	DMSO
1	*Staphylococcus aureus*	10	11.0±1.2	11.6±1.4	12.5±1.6	12.5±1.8	13±1.8	19.4±3.9	0
2	*Escherichia coli*	10	12.1±0.9	12.4±1.0	12.8±0.9	12.8±1.3	14±1.7	16.4±2.4	0
3	*Klebsiella species*	10	11.0±0.7	11.6±0.5	13.0±1.0	12.8±1.1	13.8±0.4	17.8±1.3	0
4	*Pseudomonas aeruginosa*	5	11.0±2.2	11.0±1.4	11.2±1.3	11.6±1.1	12.6±2.4	25.6±4.3	0
	Total	35	All values are expressed as mean ±SD						

Table 2: Antibacterial effect of grape seed extract against drug resistant pathogenic strains.

S No	Bacterial species	No of isolates	Concentration of grape seed extract (mg/ml)	Positive control	Negative Control
			20 mg/ml	Vancomycin	DMSO
1.	MRSA	10	4.0±6.5	17.7±0.8	0
				Amikacin	DMSO
2.	ESBL *E.coli*	10	2.7±5.7	24.9±4.0	0
3.	ESBL *Klebsiella*spp	10	0	23.6±6.6	0
	Total	30	All values are expressed as mean ±SD		

Table 3: Comparison of bactericidal effect of grape seed extract between common clinical isolates and drug resistant strains at a highest concentration of 20 mg/ml.

Bacterial species	Mean ± SD
Staphylococcus aureus	13.0±1.8
MRSA	4.0±6.5
Escherichia coli	14±1.7
ESBL *E.coli*	2.7±5.7

DISCUSSION

The bactericidal effect of grape seed extract is accounted for the presence of Stigmasterol, a sterol molecule which cause degradation of bacterial components by surface interaction and pore formation in the bacterial cell wall. It might also be related to the presence of tannins which has the ability to inactive microbial adhesions, enzymes and cell envelope transport proteins, their complexity with polysaccharide and their ability to modify the morphology of microorganisms.[5] Therefore, this observation is suggestive of the antibacterial effect of grape seed extract. In our study, grape seed extract proved to be bactericidal and was able to produce moderate zones of inhibition ranging from 11-14 mm against the common clinical isolates and 2-4 mm against the drug resistant strains with the concentrations of the extract ranging from 2 mg/ml to 20 mg/ml. The extract showed higher bactericidal effect against *E.coli* when compared to the other selected gram positive and gram negative organisms. Among the drug resistant strains MRSA and ESBL-*E.coli* were found to be susceptible to the extract and *Klebsiella* species showed no effect. A study conducted by Ashok kumar and Vijayalakshmi et al.[1] showed significant zones of inhibition ranging from 7-16 mm were produced against standard bacterial strains. Our study results obtained was found to be quite similar to their study.

Reagor L et al carried out a study to determine the effectiveness of processed grape fruit seed extract as an antibacterial agent against sixty seven distinct biotypes.[6] The results showed that the grape seed extract was consistently antibacterial against all of the biotypes tested with susceptibility zone diameters equal to or greater than 15mm in each case, suggesting that the antibacterial characteristic of grape seed extract is comparable to that of proven topical antibacterials. Su X and Howell AB et al carried out a study to determine the antibacterial effects of plant derived extracts on MRSA using commercially available grape seed extract (GSE), pomegranate polyphenols (PP) and lab prepared cranberry-proanthocyanidins against two strains of MRSA.[7] Among the three tested agents, GSE at 1 and 5 mg/ml was found to be most effective, resulting in colony reduction of both

strains after 2 hours at 37°C. According to Binith Shrestha and Srithavaj et al, the structure- activity correlation assays showed that the hydroxyl group of the phenolic compound was found to be effective against *E.coli* and the benzene ring was effective against *S. aureus*.[8] According to Al-Habeb A and Al-Saleh E et al the antibacterial effect of grape seed extract against MRSA is due to disruption of bacterial cell wall membrane in scanning and transmission electron microscopy which could be accounted to the presence of potent polyphenolics in grape seed extract.[9]

Though the study produced moderate zones of inhibition which was comparable to that of the stated references; it can further be performed with better methodologies to improve the results and to increase the effectiveness of grape seed extract against virulent strains

CONCLUSION

Increased frequency of administration and decreased effectiveness of antibiotics against common isolates has led to development of resistant strains like MRSA and ESBL producing bacteria. Hence, antimicrobial agents with minimal side effects preferably from natural products are necessary. Grape seed extract showed satisfactory antibacterial effect in this study, however the antibacterial activity could be enhanced by improving the method of extract preparation like incorporation of organic acids to grape seed extract or extraction of specific components like ethanolic and phenolic fraction using sophisticated equipments. Hence, this study may serve as a base to further researches in this field. As, grape seeds are solid byproducts of winemaking industry it could be cost effective if large scale antibacterial agents are prepared, which would be of benefit to the people belonging to lower socioeconomic strata. Once the efficacy of the grape seed extract is well established it can be used in clinical settings as an alternative or supplementary to antibiotics.

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Conflict of interest: None declared
Ethical approval: The study was approved by the Institutional Ethics Committee

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